Bespoke Strain-Level Analysis of Bacterial Genomes

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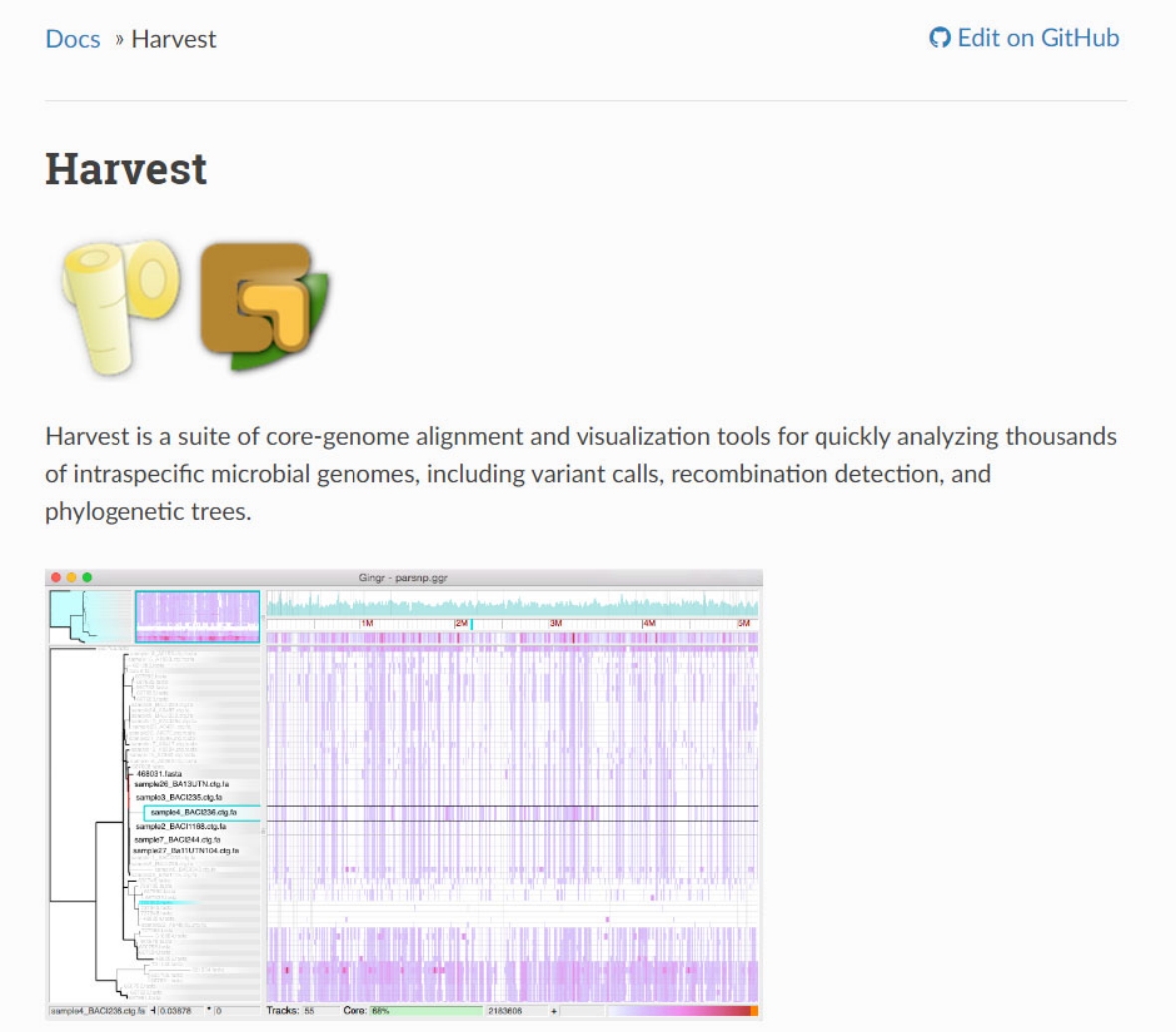
Whole-Genome Alignment

- Idea: align specifically the *shared ("core")* portion of several genomes.
- Use these aligned segments to identify phylogenetic relationships, etc…
- Visualize what exactly is similar and different…

Tools:

- Parsnp
- Mauve
- SibeliaZ
- (others…)



Whole Genome Alignment: Quick How-To with Parsnp

- Get *assembled* genomes from individual organisms
  - Isolates are nice, MAGs will do
  - Contigs are fine for this, doesn't have to be complete
  - Helps to have at least 1 high-quality, annotated reference genome
  - Useful to run QUAST to QC the assembly
- Run Parsnp:

```
contig_repo=./parsnp_contigs
parsnp_out=./parsnp_output_13
ref_genbank=./ref_assembly_GCF_008121495/Ref_ATCC_29149.gbff

parsnp -g $ref_genbank -d $contig_repo -p 15 -o $parsnp_out
```

Annotated Reference Genome (.gbff format)

Folder with 1 fasta file for each assembly (containing all contigs)

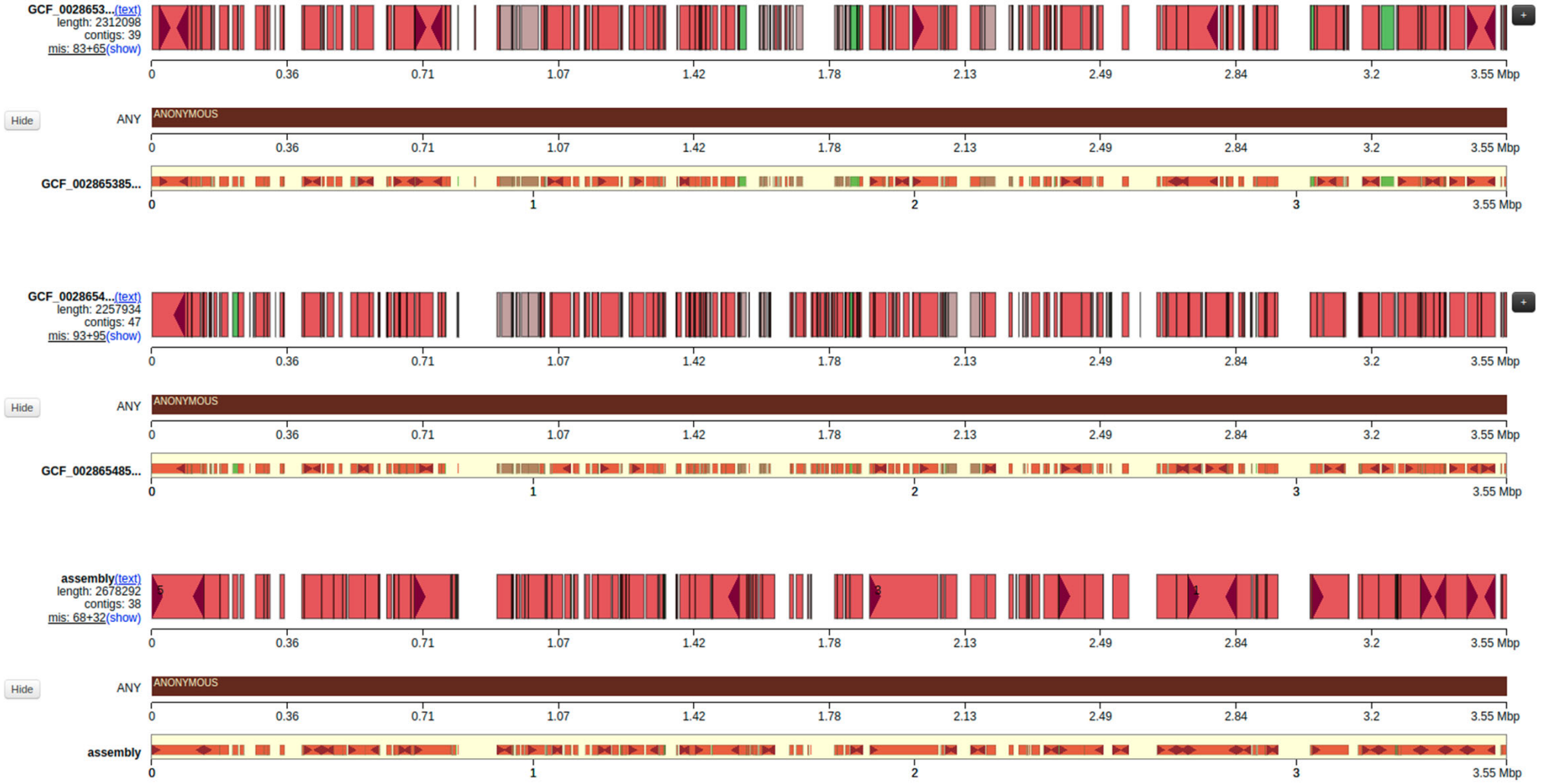
 # processors

Output folder

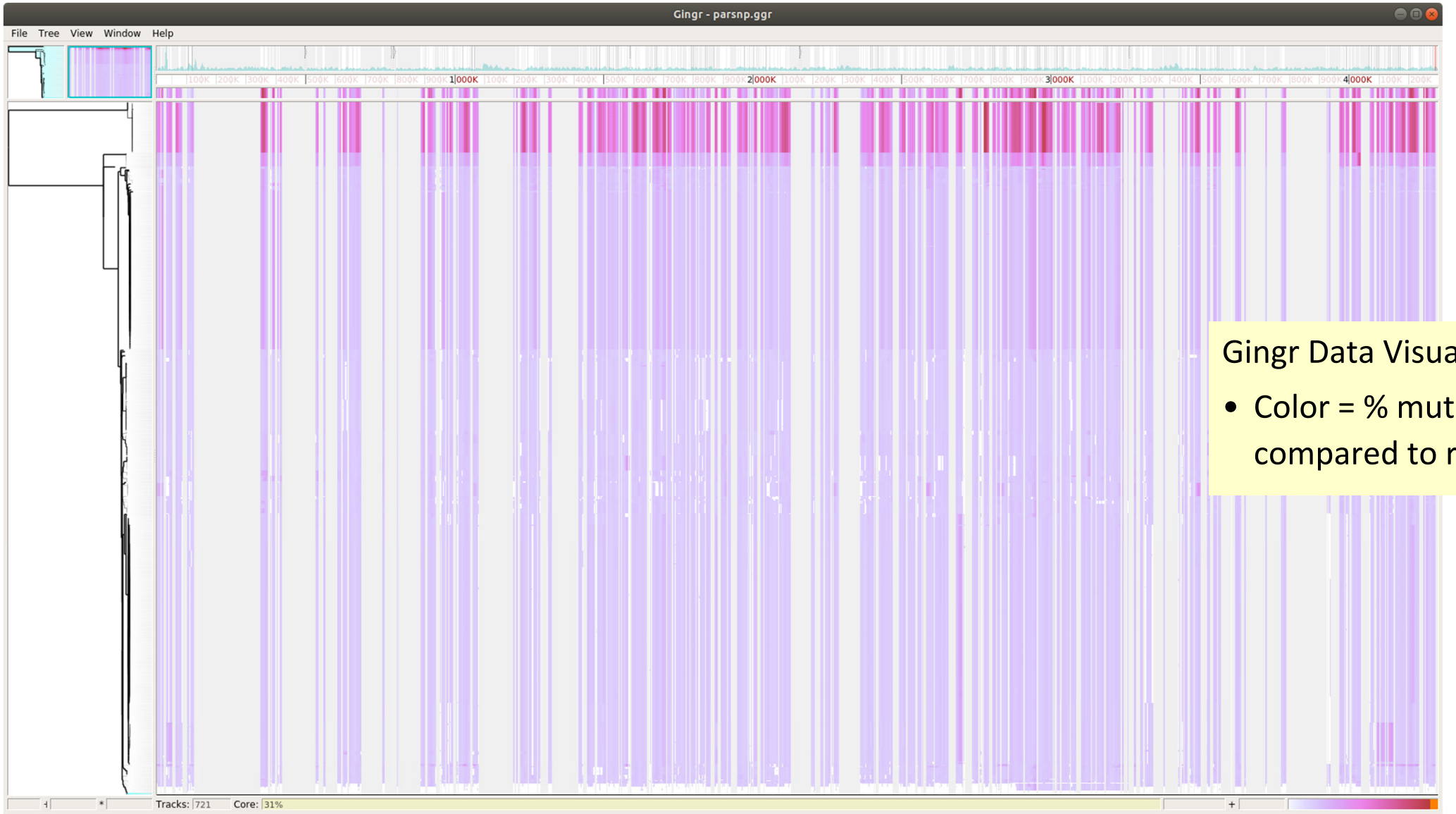
Interlude: QC-ing an Assembly with QUAST

What can we learn?

- Assembly Quality Issues?
- Issues with Reference?



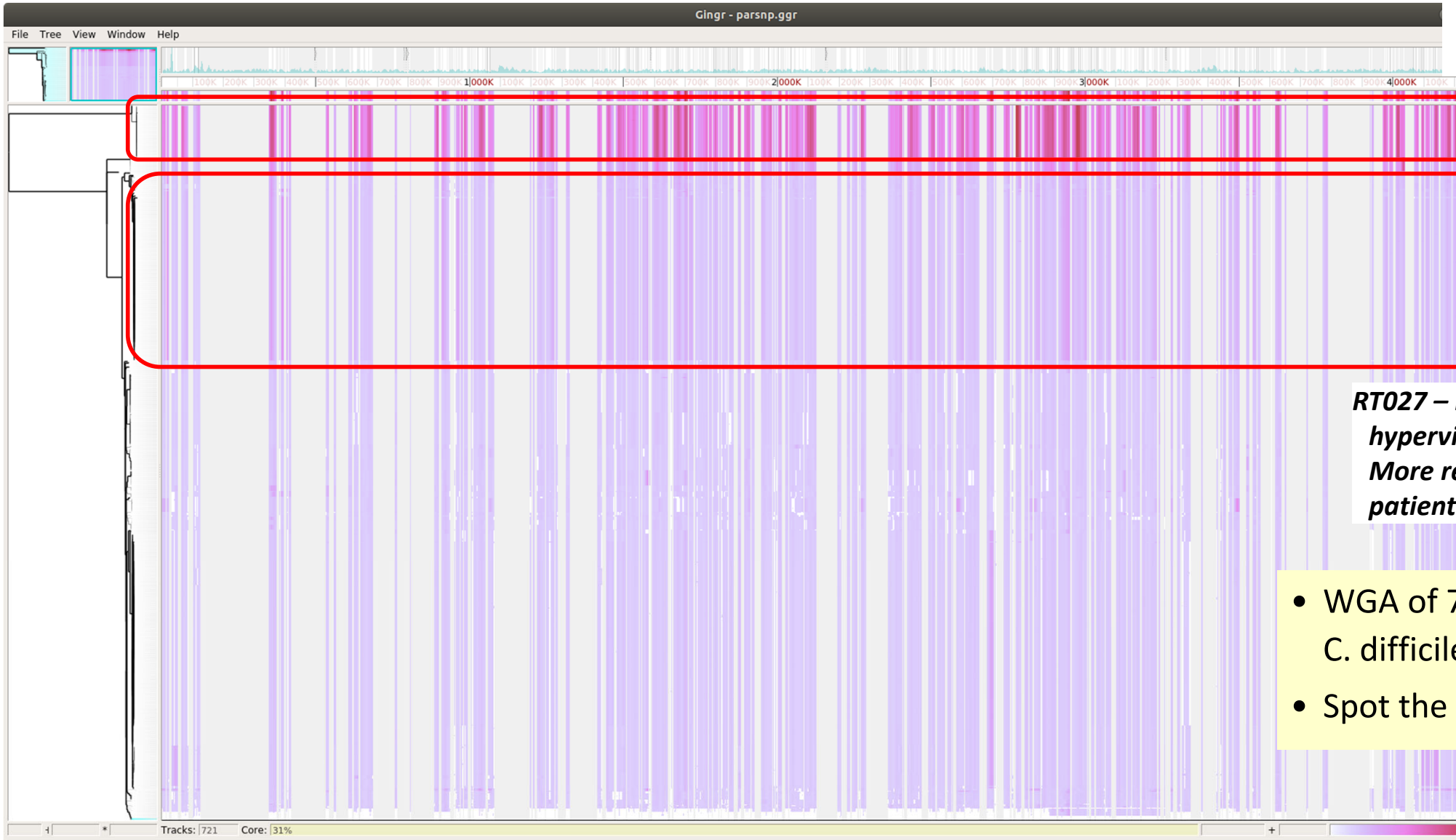
Case-Study: *C. difficile* Genomes



Gingr Data Visualization:

- Color = % mutation compared to reference

Case-Study: *C. difficile* Genomes

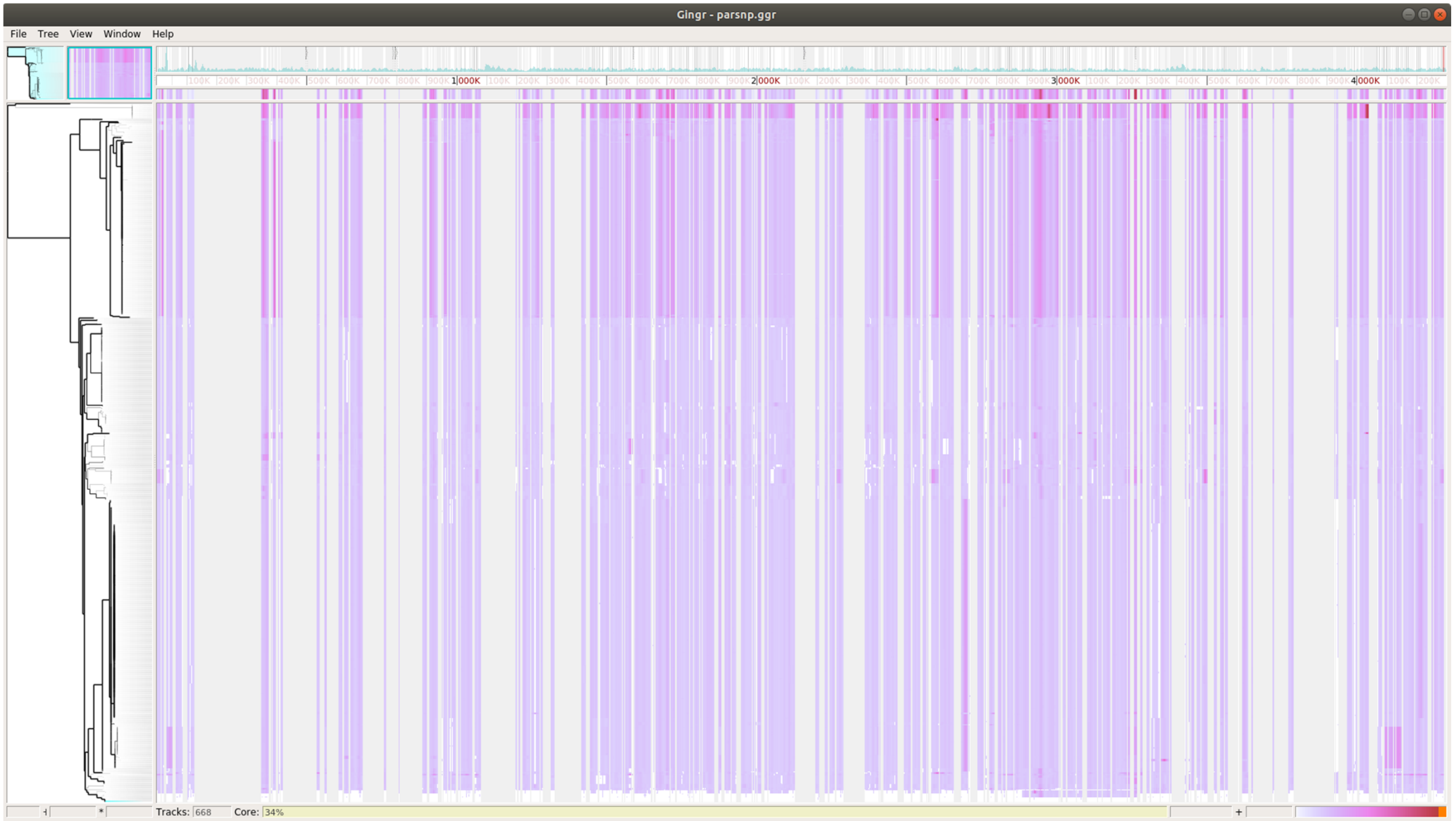


RT078 – Originated in animal host, crossed over

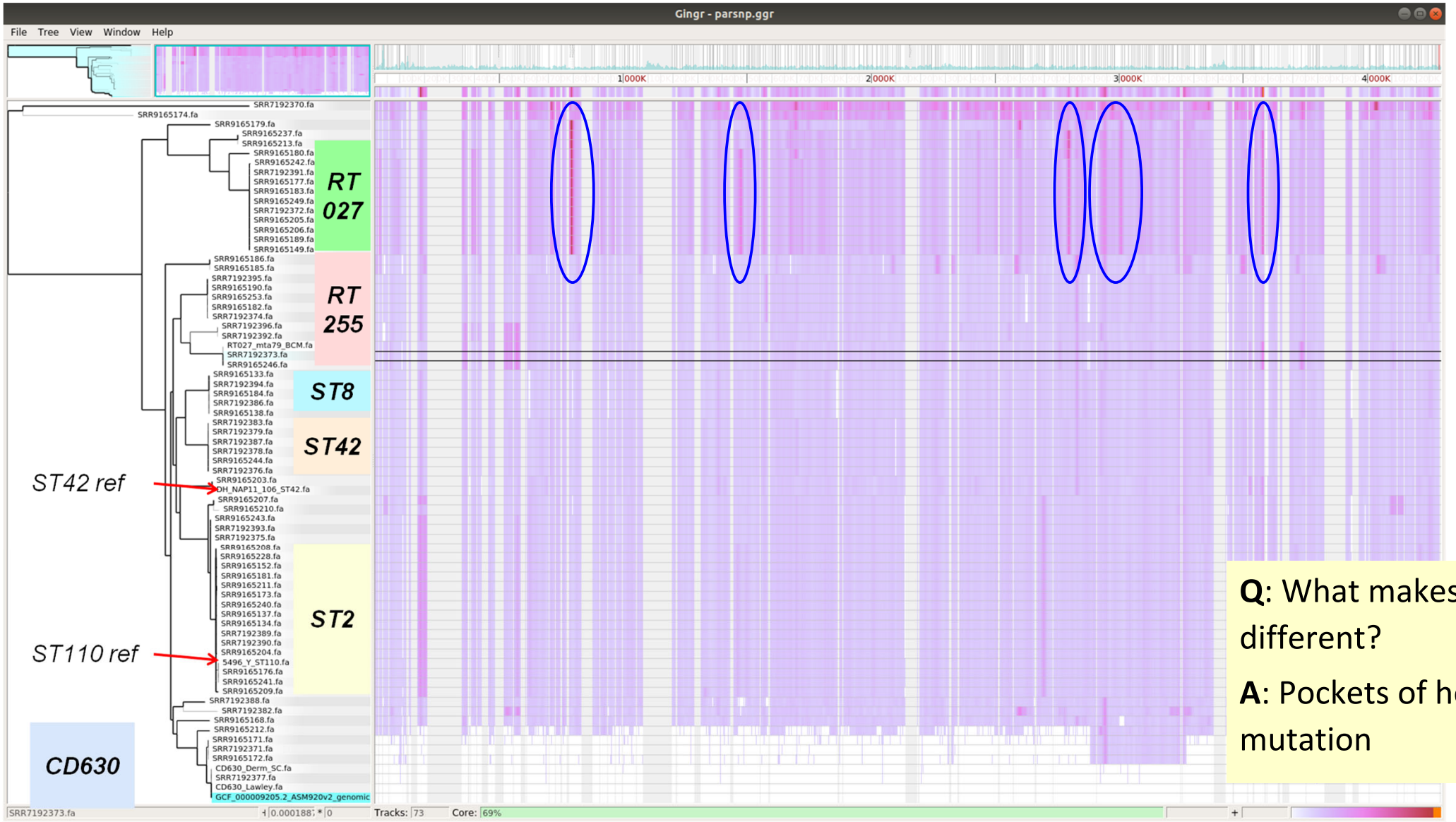
RT027 – Known hypervirulent strain. More recurrent, nastier patient outcomes.

- WGA of 720 assembled C. difficile genomes
- Spot the strains…

Case-Study: *C. difficile* Genomes (excluding RT078 samples)



Subset of Genomes w/ST annotation



Q: What makes RT027 different?

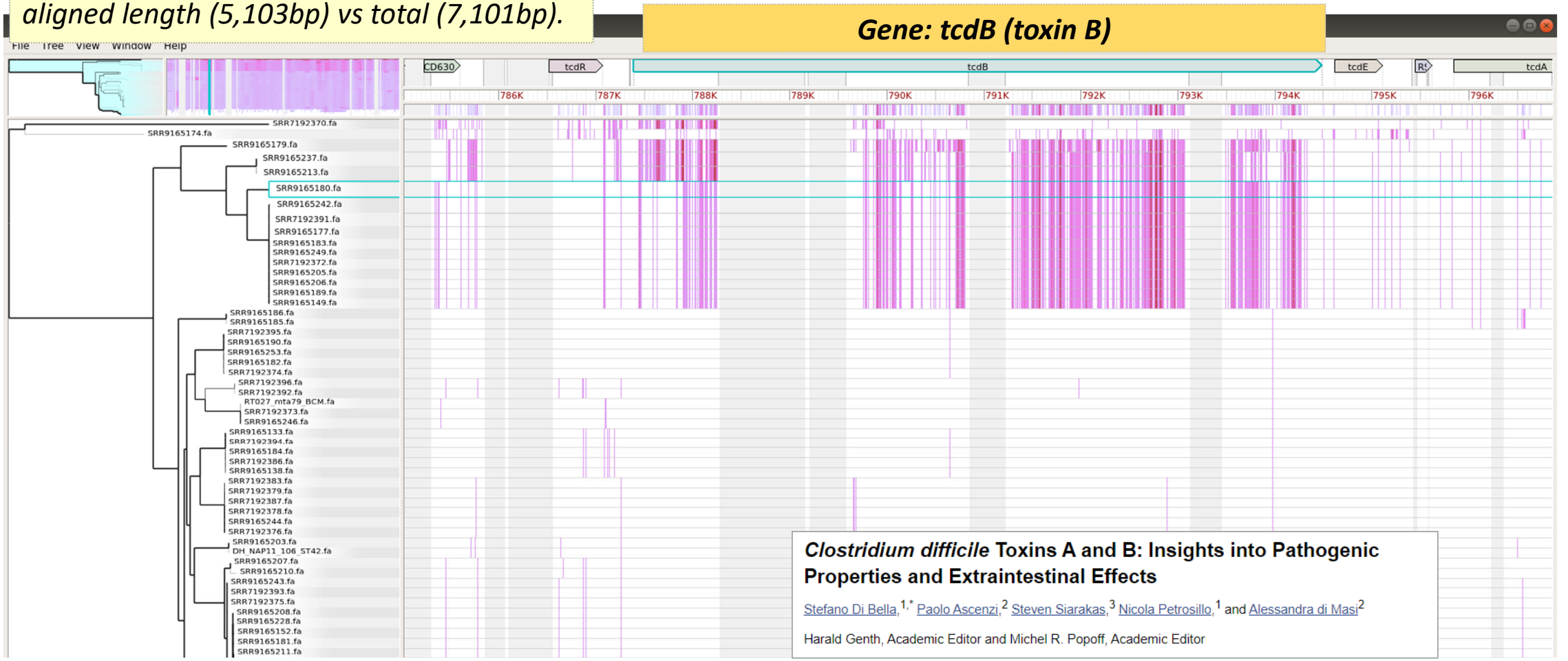
A: Pockets of heavy mutation

Digging Deeper…

- *This particular region is precisely the coding locus for Toxin B.*
- *RT027 carries a variant tcdB gene with altered function that contributes to its virulence.*

***Note**: not all of the tcdB gene was aligned by Parsnp, so this table represents the aligned length (5,103bp) vs total (7,101bp).*

Gene: tcdB (toxin B)



***Clostridium difficile* Toxins A and B: Insights into Pathogenic Properties and Extraintestinal Effects**

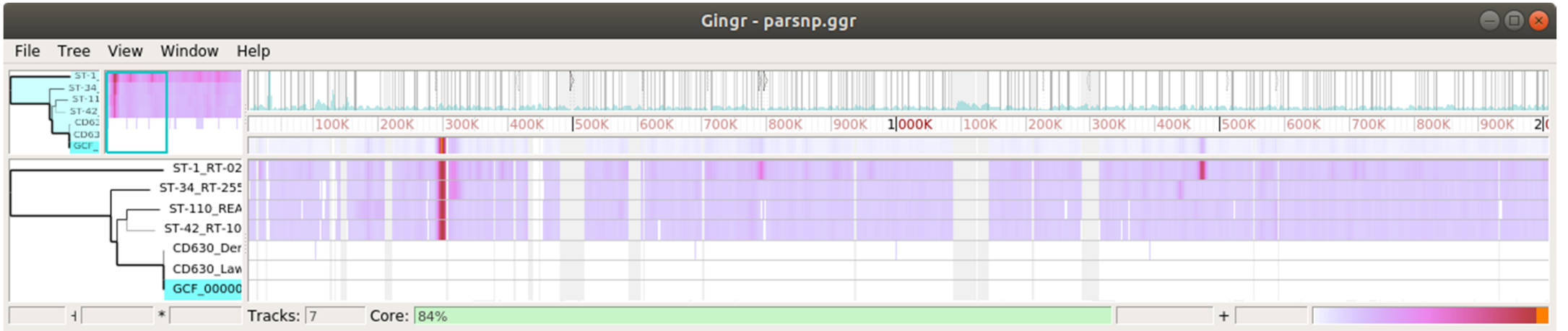
Stefano Di Bella,[1,*] Paolo Ascenzi,[2] Steven Siarakas,[3] Nicola Petrosillo,[1] and Alessandra di Masi[2]

Harald Genth, Academic Editor and Michel R. Popoff, Academic Editor

Comparing Reference Genomes for Some Strains

***Note**: RT027 is in the top row. CD630 is a lab strain used as a common reference.*

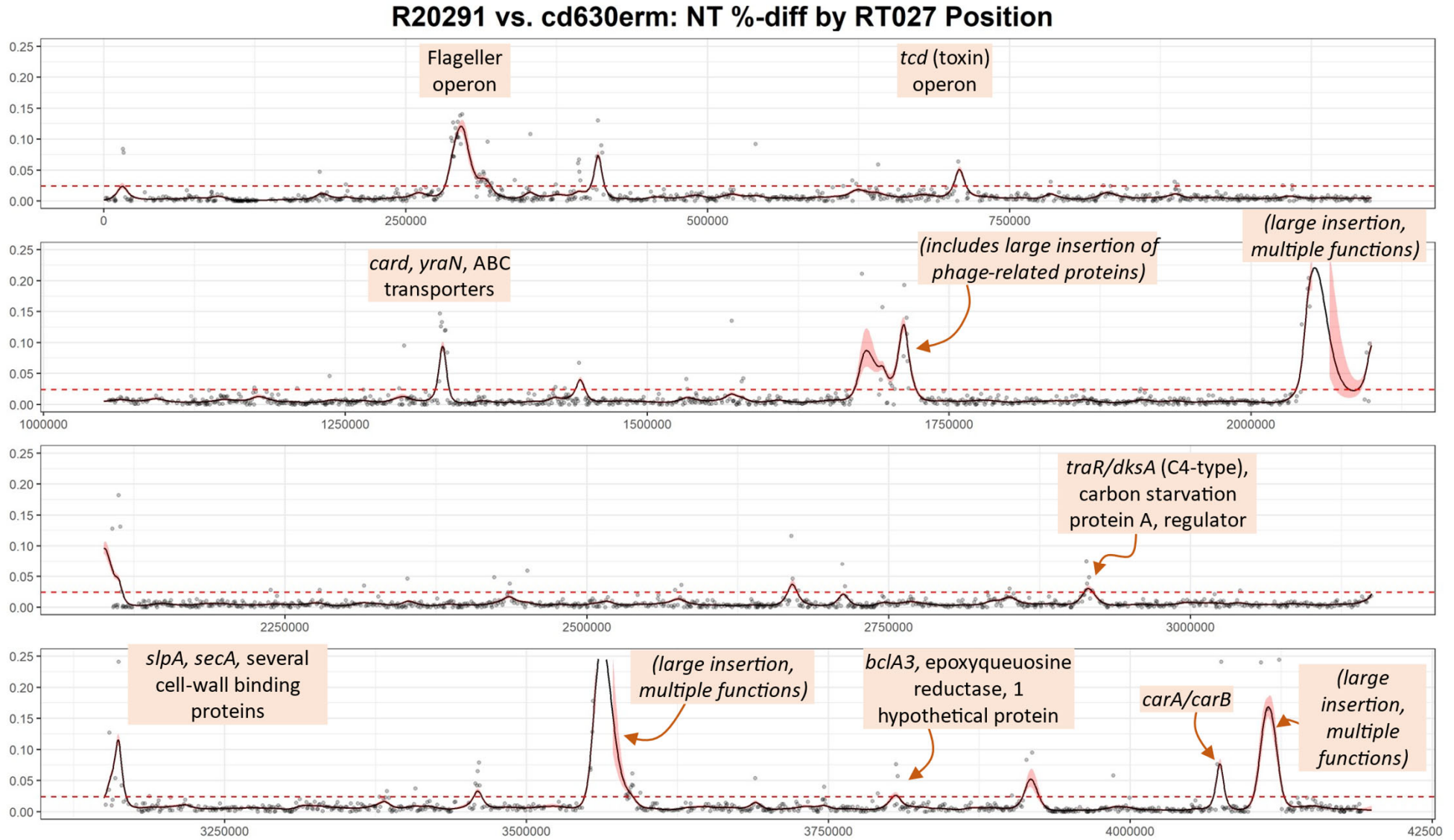
Segment 1
(positions 0-2mbp)



Segment 2
(positions 2-4mbp)

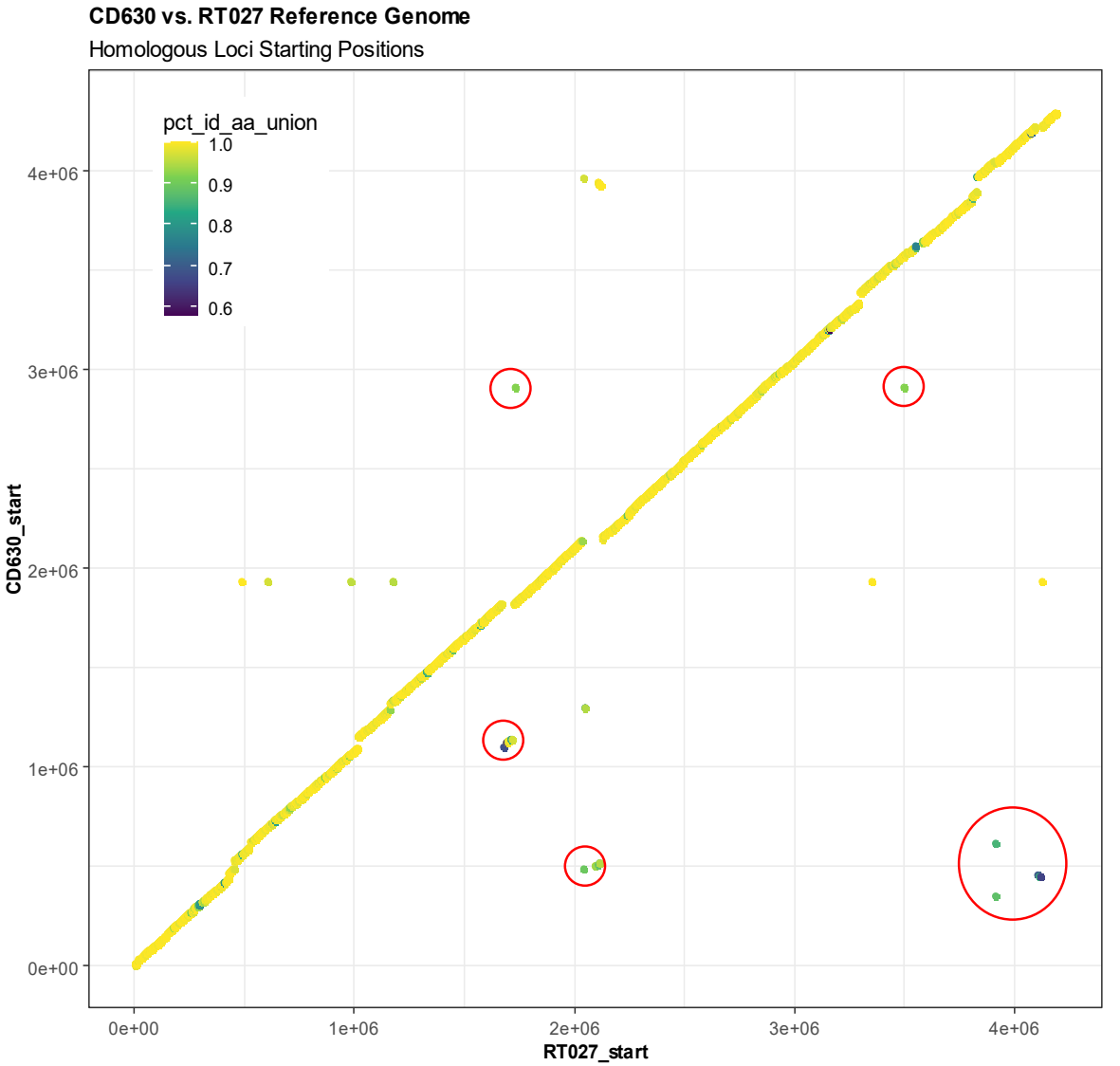
Here the mutation pockets are much clearer.

Digging Deeper Again…

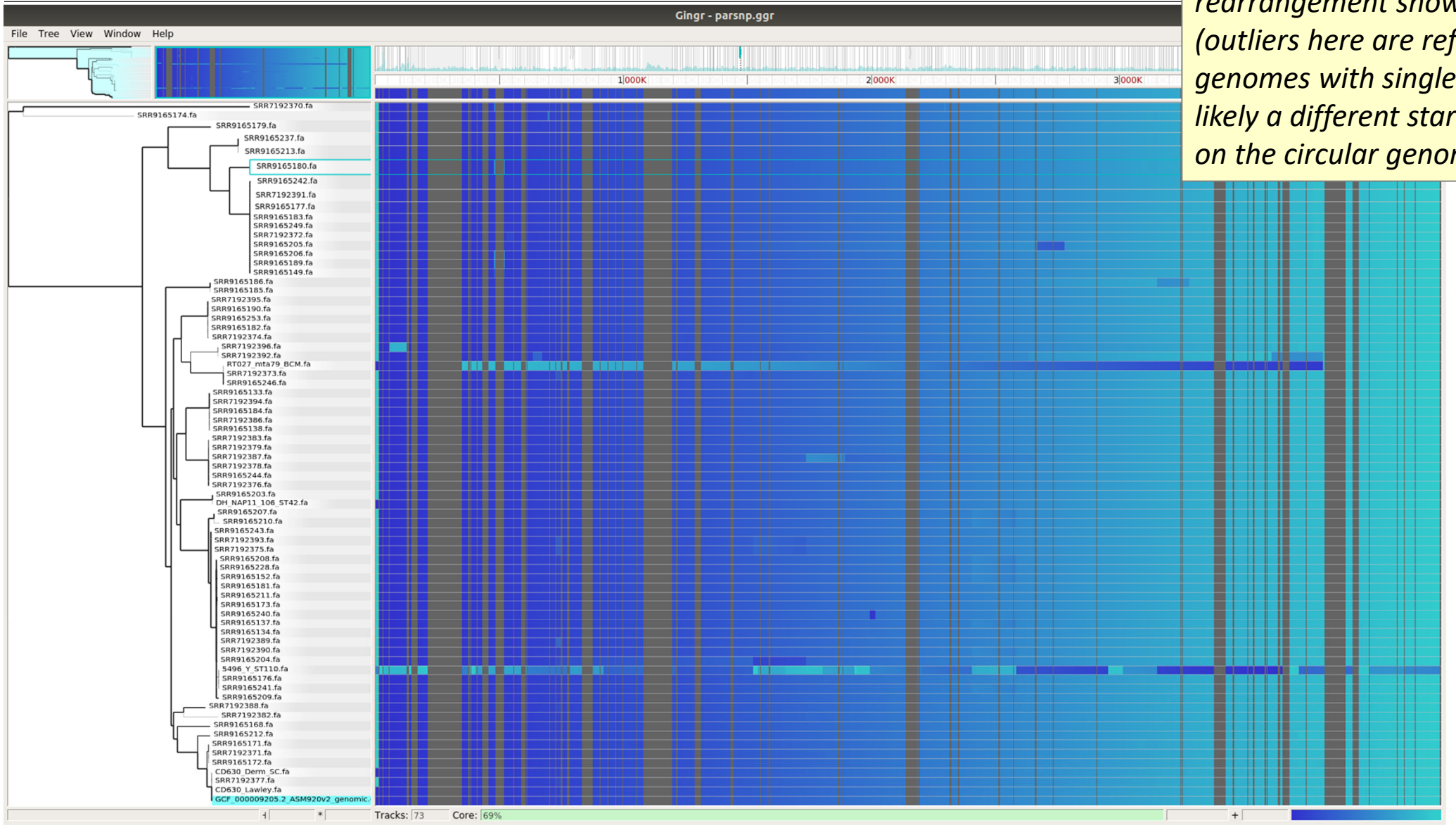


Comparing Location of Homologous Genes

- Scatter Plot
  - Each point shows position in genome for CD630 & RT027, for a single shared gene
  - Color indicates %-AA-similarity
- Despite differences, genomes are highly colinear
  - Many short indels throughout
  - No major rearrangements except a few small segments.
  - Small rearrangements coincide with locations of high-mutation



Synteny Comparison: *C. difficile* Isolates



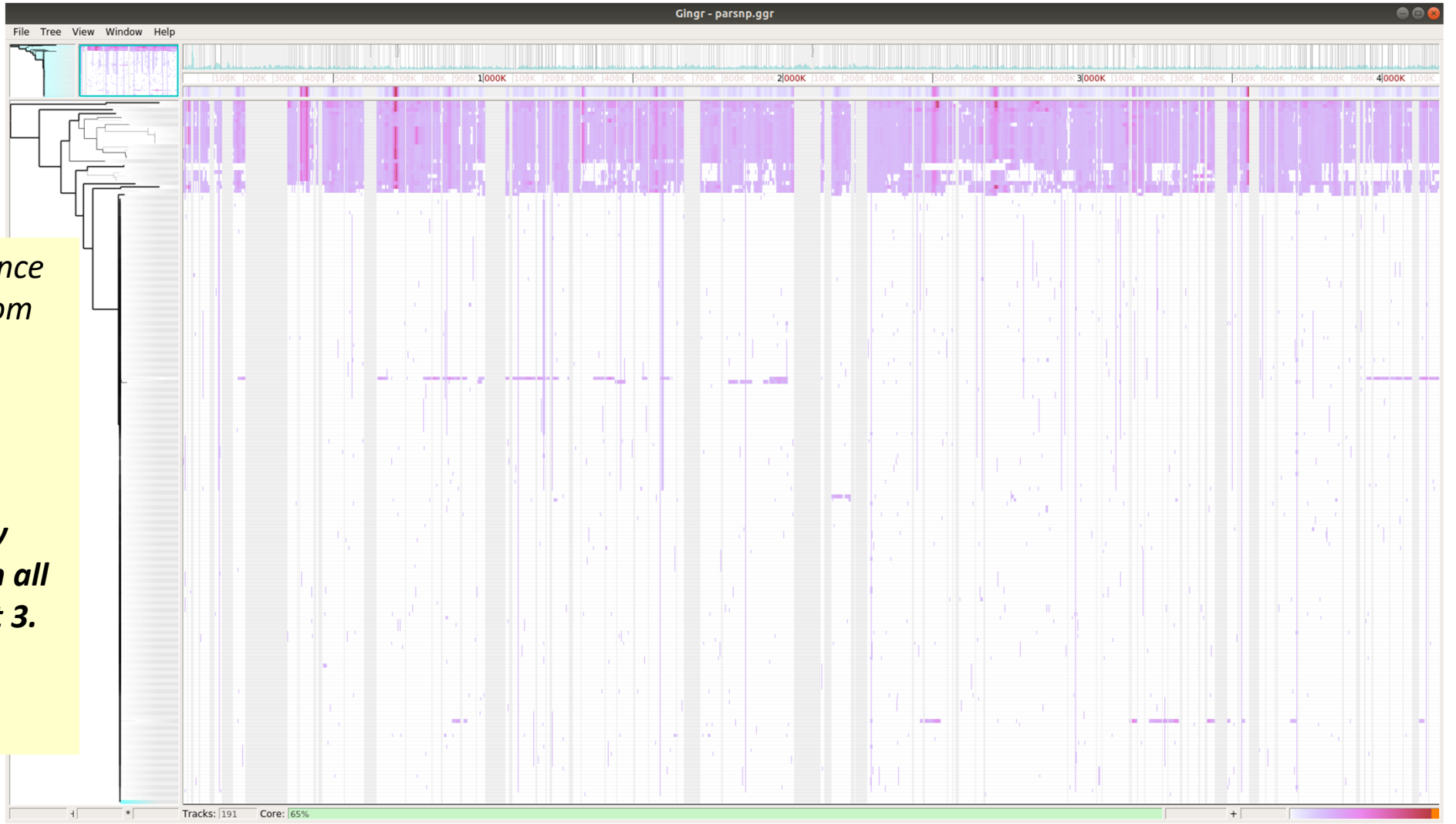
For C. diff, even across a huge number of isolates, very little rearrangement shows up (outliers here are reference genomes with single contig, likely a different starting point on the circular genome.)

Alignment of RT027 isolates (and near relatives) to RT027 ref.

Does the RT027 Reference match the genomes from the clinic?

…Yes

- ***Very little to see, very high match level with all RT027 isolates except 3.***



Another Case Study: *R. gnavus* Isolates from IBD Patients

14 Genomes:

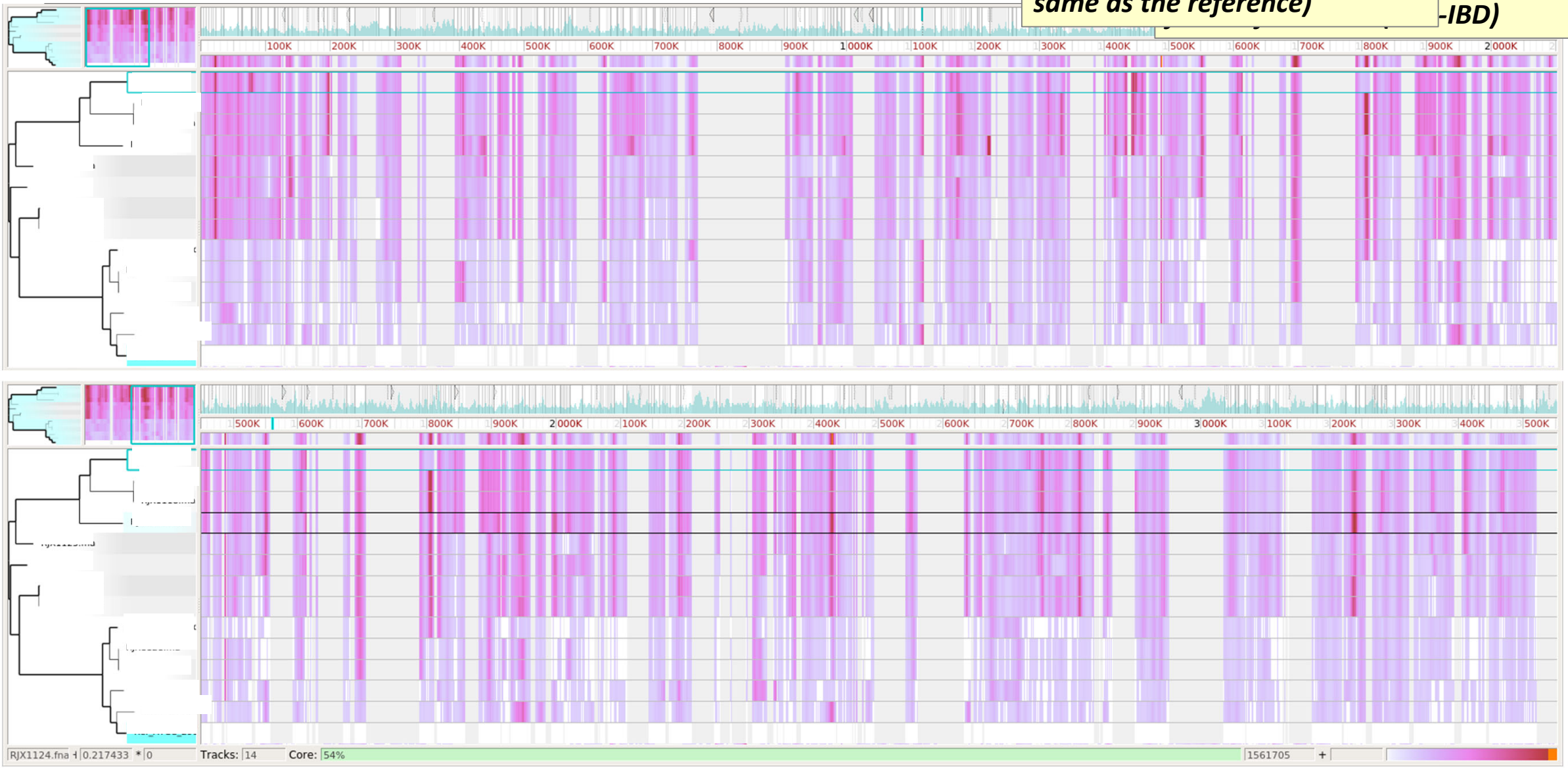
- Reference: ATCC 29149 (RefSeq GCF_008121495)
- ATCC 29149 *de novo* assembly (by me)
- ATCC 35913 (GenBank GCA_900036035)
- 12 Genomes from Hall et al. (2017) (table at right)

RJX1118*	Stool from infant treated with antibiotics
RJX1119*	Stool from infant treated with antibiotics
RJX1120*	Biopsy from IBD patient
RJX1121*	Biopsy from IBD patient
RJX1122*	Biopsy from IBD patient
RJX1123*	Biopsy from IBD patient
RJX1124*	Biopsy from IBD patient
RJX1125*	Biopsy from IBD patient
RJX1126*	Biopsy from IBD patient
RJX1127*	Biopsy from IBD patient
RJX1128*	Stool from IBD patient

Another Case Study: *R. gnavus* Isolates from IB

Game 2 : Spot the 2nd ATCC 29149 gnome (supposedly the same as the reference)

enomes -IBD)



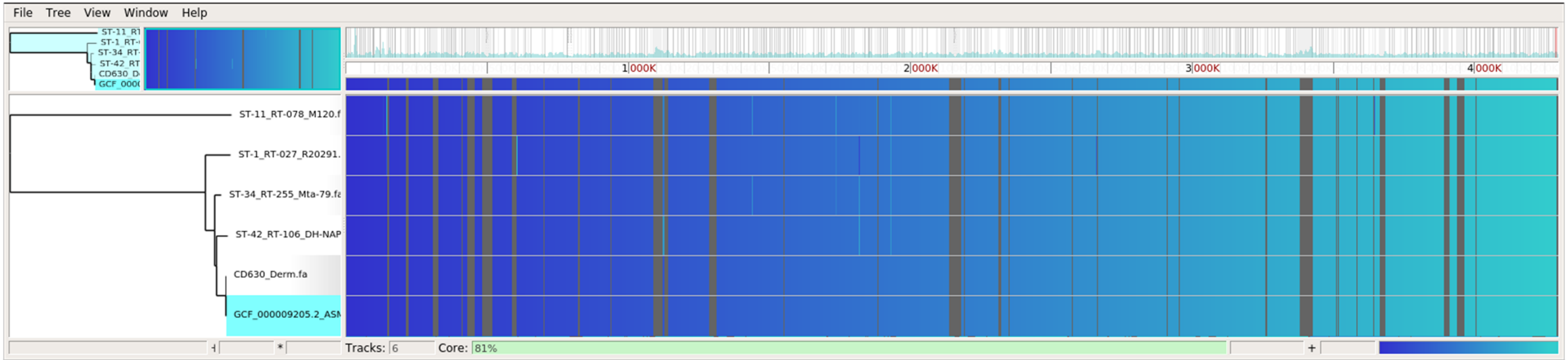
R. gnavus strain-level phylogenetic signal is a mess

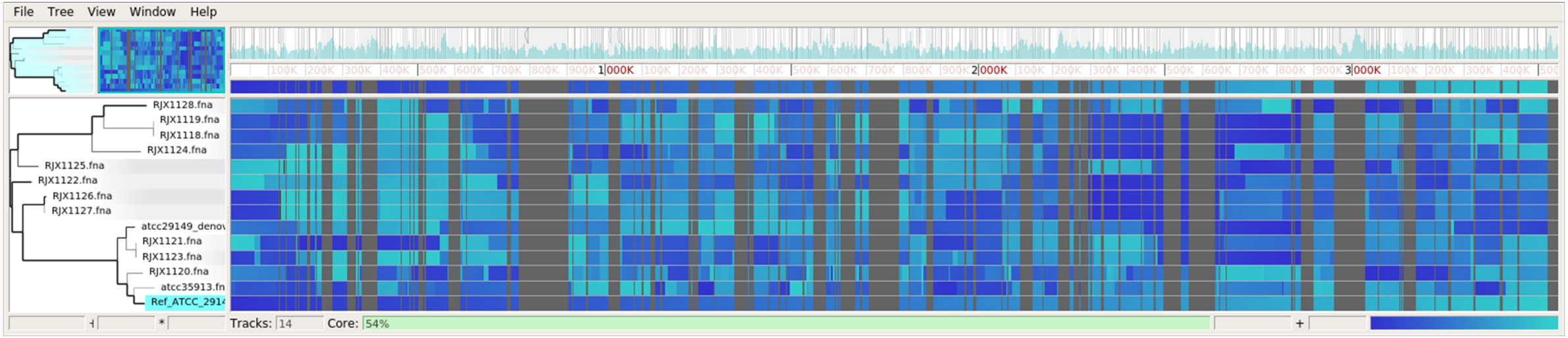
Depending on the operon, the phylogenetic appearance is completely different
⇒ ***Highly plastic genome***

This does NOT show genome rearrangements (synteny)

Synteny Comparison: *R. gnavus* & *C. difficile*

These two organisms have very different types of genome plasticity.





Conclusions

- Whole-genome alignment will give a detailed comparison specifically of the *core* genome
  - Maybe also auxiliary genes (*pan*-genome)
- Visualization can get you up close and personal with the data
  - (This statement applies to almost everything, not just genomes)
- Strains can differ from one another in weird ways.
  - Selective mutation at points of interest
  - Gene gain/loss depending on environment
  - Genome-wide phylogenetic signal vs. Locus-specific signal
  - Etc…?

Special Thanks To:

- The Treangen Lab (Rice)
  - Todd Treangen
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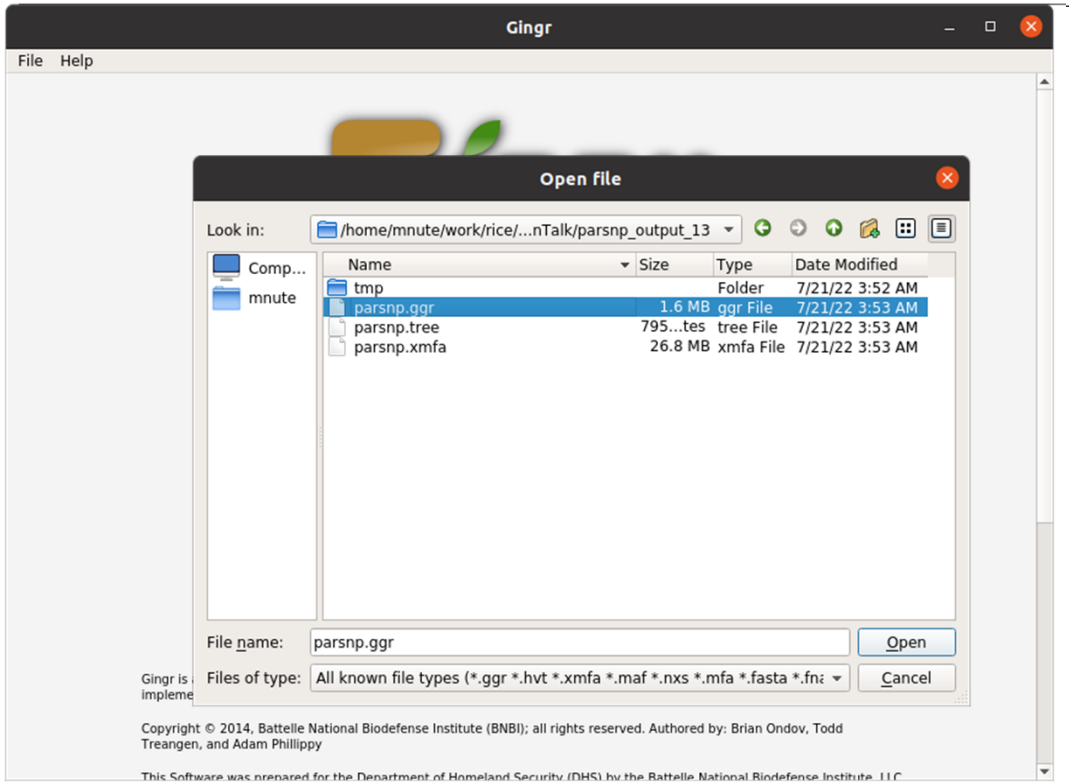
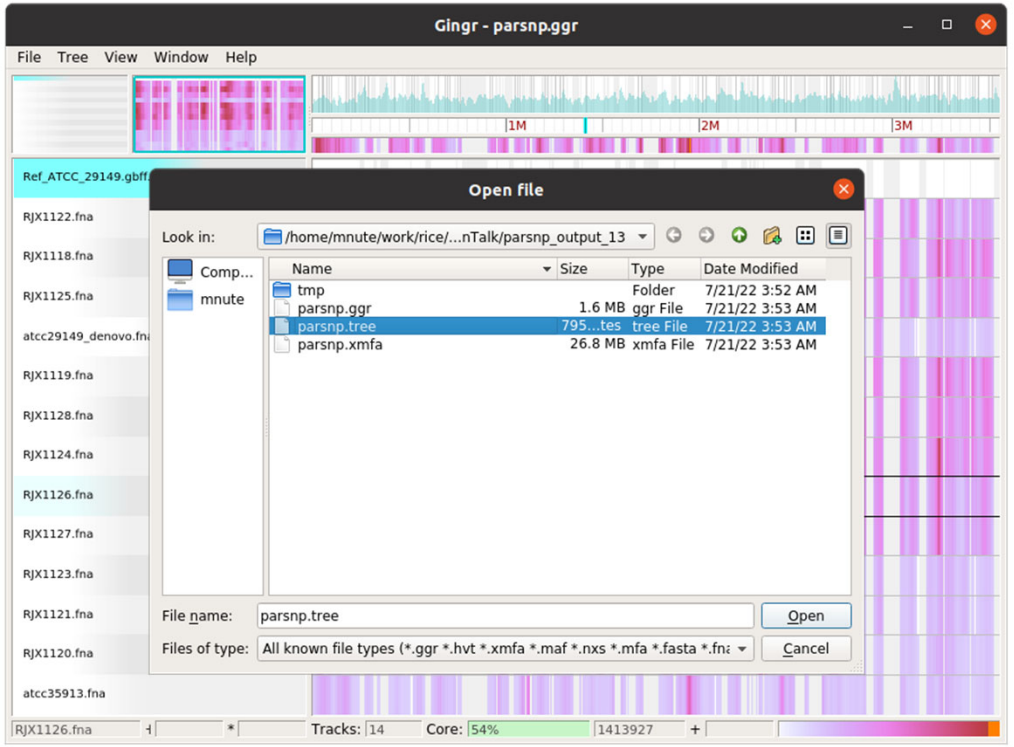
  - Yilei Fu
  - Advait Balaji
- The Savidge Lab (Baylor College of Medicine)
  - Qinglong Wu
  - Charlie Seto
- Taylor Reiter (for the *R. gnavus* idea)

Appendix: Quick How-to with Gingr (1 of 2)



*1.) Open the *.ggr file created in the parsnp output folder.*



*2.) Once it is open, go back to the "Open" dialogue and open the *.tree file in the same folder.*

Appendix: Quick How-to with Gingr (2 of 2)

3.) This will give you the standard Gingr view. Other options to re-root the tree or to switch to Synteny view are available under the "Tree" and "View" menus.